PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)
	) Group Art Unit: 1652
Xiao and Gedrich	)
	) Examiner: D. Ramirez
Serial No. 09/879,792	)
	) Docket No. 002973.00035
Filing Date: June 13, 2001	)

For: **REGULATION OF HUMAN TRANSMEMBRANE SERINE PROTEASE**

DECLARATION UNDER RULE 131

We, Yonghong Xiao and Richard Gedrich, hereby declare:

1. We are the named inventors of the subject matter claimed in the application referenced above.

2. Prior to March 19, 2001, we conceived of and reduced to practice molecules comprising the polynucleotide sequence disclosed as SEQ ID NO:11 and the amino acid sequence disclosed as SEQ ID NO:12 in the above-referenced application.

3. The nucleotide sequence of a contig identified as SEQ ID NO:11 in serial number 60/211,224 was extended by amplification and alignments with mouse EST and human genomic sequences. The nucleotide sequence of an extended contig containing a portion of the sequence of SEQ ID NO:11 in serial number 09/879,792 was identified. The nucleotide sequence of the extended contig is shown on the attached copy of page 3 of laboratory notebook RB55202 and identified as "147.33 contig" (Exhibit A).

4. The Basic Local Alignment Search Tool (BLAST) algorithm was used to extend the 5' end sequence of the 147.33 contig. The BLAST algorithm aligned human expressed sequence tags (ESTs) having sequence similarity with the 5' sequence of the 147.33 contig. The results of the BLAST searches were recorded on pages 20-23 of laboratory notebook RB55202. Copies are provided as Exhibit B.

5. An EST, BE280394, was found to overlap the 5' sequence of the 147.33 contig. The BE280394 EST sequence was recorded on page 24 of laboratory notebook RB55202. A copy

of page 24 is provided as Exhibit C. The alignment of EST BE280394 with the 5' end of the contig is shown in second column of page 20, the first column of page 21, and the first lines of the second column of page 21 (Exhibit B).

6. To confirm that the 147.33 contig extended at its 5' end with the sequence of EST BE280394 included the sequence of a full-length gene, the extended 147.33 contig was translated by computer into an amino acid sequence. The translation of the extended contig in all possible open reading frames associated with the extended sequence was recorded on pages 27 and 28 of laboratory notebook RB55202. Copies are provided as Exhibit D. The extended contig was confirmed to contain a full-length gene because its translation into an amino acid sequence identified a start (methionine) residue at its amino terminal end and the start codon is in frame with a predicted amino acid sequence encoded by the 147.33 contig. The translated sequence identified as the one encoding the protein shown in SEQ ID NO:12 is underlined and begins "MERDSH." The hand-written notes at the top of page 29 of laboratory notebook RB55202 identify the extended contig sequence as the sequence of the "147" full-length gene. A copy of page 29 is included in Exhibit D.

7. Primers capable of amplifying the 147 full-length open reading frame were ordered based on the nucleotide sequence that encodes the translated protein. The oligonucleotide sequences ordered were recorded on page 30 of laboratory notebook RB55202, a copy of which is provided as Exhibit E.

8. Human placenta cDNA was amplified using these primers to obtain the molecule in human genomic DNA that contains the 147 full-length open reading frame. The primers used to perform the amplification and the conditions of the amplification reaction were recorded on page 40 of laboratory notebook RB55202. A copy of page 40 is provided as Exhibit F.

9. The amplification product obtained from this reaction was the predicted size for the full-length open reading frame. An agarose gel showing the amplification product obtained using the primers is shown in a photograph on page 41 of laboratory notebook RB55202. A copy of page 41 is provided as Exhibit G.

10. The amplification product was extracted from the gel using the "QIAquick Gel Extraction Kit Protocol" and cloned into a vector sold by Invitrogen and referred to the "TA vector." The protocol used to clone the amplification product (full-length open reading frame)

into the vector was recorded on pages 41 and 42 of lab notebook RB55202. Copies of pages 41 and 42 are provided as Exhibits G and H, respectively.

11. The vector containing the amplification product was transformed into *E. coli* TOP10 cells, as described on page 42 of laboratory notebook RB55202 (Exhibit H).

12. The vector was extracted from the transformed *E. coli* and digested with restriction enzyme *Eco*RI to confirm the presence of the amplification product in the vector. The protocol "QIAprep Spin Miniprep Kit Protocol," which was used to obtain the vector DNA from *E. coli*, was recorded on page 1 of laboratory notebook RB55846. A copy of page 1 is included in Exhibit I. The conditions under which the vector DNA was digested and a picture of the agarose gel showing the results of restriction enzyme digestion are provided on page 7 of laboratory notebook RB55846. A copy of this page is included in Exhibit I. The agarose gel on the laboratory notebook page confirms the presence of the 147 full-length open reading frame in each vector but for one.

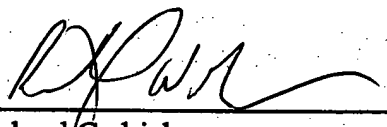
13. Vectors containing the amplification product were sequenced. The composition of the tubes containing the vector and primers used in the sequencing reaction were recorded on page 8 of laboratory notebook RB55846. A copy of page 8 is provided as Exhibit J.

14. The nucleotide sequence obtained for amplification product cloned into the vector was recorded on pages 15-17 of laboratory notebook RB55846. Copies of pages 15-17 are provided as Exhibit K. The recorded nucleotide sequence is identical to the sequence identified as SEQ ID NO:11 in application serial number 09/879,792.

15. The nucleotide sequence of the amplification product was translated into an amino acid sequence. This translated amino acid sequence was recorded on pages 17-18 of laboratory notebook RB55846. Copies of pages 17-18 are provided as Exhibit L. The recorded amino acid sequence is identical to the sequence identified as SEQ ID NO:12 in application serial number 09/879,792.

16. The dates on each of the attached Exhibits have been redacted. All of the work described in paragraphs 3 through 15 was performed in the United States and completed before March 19, 2001.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States; and further that false statements made willfully may jeopardize the validity of any patent issuing on an application in which the false statements were made.

Date	Yonghong Xiao
6/5/03	
Date	Richard Gedrich

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that false statements made willfully are punishable by fine, imprisonment, or both a fine and imprisonment under Section 1001 of Title 18 of the United States; and further that false statements made willfully may jeopardize the validity of any patent issuing on an application in which the false statements were made.

6/9/03
Date

Yonghong Xiao

~~6/9/03~~
Date

Richard Gedrich

BAYER CORPORATION

Note Book 3
RB55202

SUBJECT 147.33.contig2 Sequence (Nucleotide)

EXHIBIT
tabbies
A

147.33.contig2
[Strand]

```
1     GAATTCCCCC TTGCTTTCAG AATGCATCTC CAGCAAGAAC
41    ACTTTCAGCT GGAGCATCTC CAGCCCAGGT ATCTCCAGCT
81    GGGACACCTC CAGCCCCGGC ATCTCCAGCC CAGGCATCTC
121   CAGCCCAGGC ATCTCCAGCT GGGACACCTC CGGGCCGGGC
161   ATCTCCAGCC CAGGCATCTC CAGCTGGTAC AGCTCCAGCC
201   CGGGCATCTC CAGGCCGGGC ATCTCCAGCC CAGCCATCTC
241   CAGCCCAGGC ATCTCCGGCT CTGGCATCAC TTTCCAGGTC
281   CTCATCCGGC AGGTCATCAT CCGCCAGGTC AGCCTCGGTG
321   ACAACCTCCC CAACCAGACT GTACCTTGTT AGAGCAACAC
361   CAGTGGGGGC TGTACCCATC CGATCATCTC CTGCCAGGTC
401   AGCACCAGCA ACCAGCGCCA CCAGCCAGAG GCCAGGTACC
441   AGCCTGCCCA ACTTCACCTG GCGGGAGGGC CAGAAGCACC
481   TACCGCTCAT CGGGTGCGTG CTCCTGCTCA TTGCCCTGGT
521   CGTTTCGGTC ATCATCCTCT TCCAGTTCTC CCAGGGCCAC
561   ACAGGGATCA GGTACAAGGA GCAGAGGGAC AGCTGTCCCA
601   AGCACGCTGT TCGCTGTGAC GGGCTGGTCC ACTGCAAGCT
641   GAAGAGTGAC CAGCTGGCCT CCTGAGGTT TGACTGGGAC
681   AAGTCTCTGC TTAAAATCTA CTCTGGGTCC TCCCATCAGT
721   GGCTTCCCAT CTGTAGCAGC AACTGGAATG ACTCCTACTC
761   ACAGAAGACC TGCCAGCAGC TGGGTTTCGA GAGTGCTGAC
801   CCGACAACCG AGGTTGCCCA CAGGGATTTT GCCAACAGCT
841   TCTCAATGTT GAGATACAAC TCCACCATCC AGGAAGCCT
881   CCACAGGTCT GAATGCCCTT CCCAGCCGTA TATCTCCCTC
921   CAGTGTTCCC ACTCCGGACT CAGCGCCATG ACCGGGCGCA
961   TCGTGGGAGG GCCGCTCGCC TCCGATAGCA AGTCGCCTTC
1001  GCAAGTGAGT CTGCACTTCG GCACCACCCA CATCTGTCGA
1041  GGCACGCTCA TTGACGCCCA GTGGGTGCTC ACTGCCCCCC
1081  ACTGCTTCTT CCTGACCCGG GAGAAGGTCC TGCAGGGCTG
1121  GAAGCTCTAC GCGGGCACCA GCAACCTGCA CCAGTTGCCT
1161  CAGCCAGCCT CCATTGCCGA CATCATCATC AACAGCAATT
1201  ACACCGATGA GGAGGACGAC TATGACATCG CCCTCATGCG
1241  GCTGTCCAAG CCCCTGACCC TGTCCGGTCA CATCCACCCT
1281  GCTTGCCTCC CCATGCATGG ACAGACCTTT AGCCTCAATG
1321  AGACCTGCTG GATCACAGGC TTTGGCAAGA CCACGGAGAC
1361  AGATGACAAG ACATCCCCCT TCCTCCGGGA GGTGCAGGTC
1401  AATCTCATCG ACTTCAAGAA ATGCAATGAC TACTTGGTCT
1441  ATGACAGTTA CCTTACCCCA AGGATGATGT GTGCTGGGGA
1481  CCTTCGTGGG GGCAGAGACT CCTGCCAGGG AGACAGCGGC
1521  GGGCCTCTTG TCTGTGAGCA GAACAACCGC TGGTACCTGC
1561  CAGGTGTCAC CAGCTGGGGC GCAGCCTGTG GCCAGACAAA
1601  CAAACCTGGT GTGTACACCA AAGTGACAGA AGTTCTTCCG
1641  TGGATTTACA CCAACATGGA GAGCGAGGTG CCATTCAGAA
1681  AATCCTAACC AGCTGGCCTG CTGCTCTGCA CAGCACCGGC
1721  TGCTGTGACT AAGGGCGAAT TC
```

LifeTools Retrieve FASTA Sequence

LifeTools — Retrieve FASTA Sequences

Project ID: ccolLSPI47 — Project Name: TM Serine Proteases
Created: — Created By: Gedrich, Richard
Modified: — Modified By: Gedrich, Richard
Status: OPEN — Current User: Gedrich, Richard (WRITE)
Today's Date: — Num Seqs: 26

```
>ccolLSPI47|147.33.49.26contig2:1
GAATTCCCCCTTGCTTTCAGAATGCATCTCCAGCAAGAACACCTTCAGCTGGAGCATCTCCAGCCCAGGTATCTCCAGCT
GGGACACCTCCAGCCCCGGCATCTCCAGCCCAGGCATCTCCAGCCCAGCATCTCCAGCTGGGACACCTCCGGGCCGGGC
ATCTCCAGCCCAGGCATCTCCAGCTGGTACAGCTCCAGCCCGGGCATCTCCAGCCCAGCCATCTCCAGCCGGCACCTC
CAGCCCAGGCATCTCCGGCTCTGGCATCACTTTCCAGGTCCTCATCCGGCAGGTCATCATCCGCCAGGTCAGCCTCGGTG
ACAACCTCCCCAACCAGACTGTACCTTGTTAGAGCAACACCAGTGGGGGTGTACCCATCCGATCATCTCCTGCCAGGTC
AGCACCAGCAACCAGCGCCACCAGCCAGAGGCCAGGTACCAGCCTGCCCAACTTCACCTGGCGGGAGGGCCAGAAGCACC
TACCGCTCATCGGGTGCGTGCTCCTGCTCATTGCCCTGGTCGTTTCGGTCATCATCCTCTTCCAGTTCTCCCAGGGCCAC
ACAGGGATCAGGTACAAGGAGCAGAGGGACAGCTGTCCCAAGCACGCTGTTCGCTGTGACGGGCTGGTCCACTGCAAGCT
GAAGAGTGACCAGCTGGCCTCCTGAGGTTTGACTGGGACAAGTCTCTGCTTAAAATCTACTCTGGGTCCTCCCATCAGT
GGCTTCCCATCTGTAGCAGCAACTGGAATGACTCCTACTCAGAAGACCTGCCAGCAGCTGGGTTTCGAGAGTGCTGAC
CCGACAACCGAGGTTGCCCACAGGGATTTTGCCAACAGCTTCTCAATGTTGAGATACAACTCCACCATCCAGGAAGCCT
CCACAGGTCTGAATGCCCTTCCCAGCCGTATATCTCCCTCCAGTGTTCCCACTCCGGACTCAGCGCCATGACCGGGCGCA
TCGTGGGAGGGCCGCTCGCCTCCGATAGCAAGTCGCCTTCGCAAGTGAGTCTGCACTTCGGCACCACCCACATCTGTCGA
GGCACGCTCATTGACGCCCAGTGGGTGCTCACTGCCCCCCACTGCTTCTTCCTGACCCGGGAGAAGGTCCTGCAGGGCTG
GAAGCTCTACGCGGGCACCAGCAACCTGCACCAGTTGCCTCAGCCAGCCTCCATTGCCGACATCATCATCAACAGCAATT
ACACCGATGAGGAGGACGACTATGACATCGCCCTCATGCGGCTGTCCAAGCCCCTGACCCTGTCCGGTCACATCCACCCT
GCTTGCCTCCCCATGCATGGACAGACCTTTAGCCTCAATGAGACCTGCTGGATCACAGGCTTTGGCAAGACCACGGAGAC
AGATGACAAGACATCCCCCTTCCTCCGGGAGGTGCAGGTCAATCTCATCGACTTCAAGAAATGCAATGACTACTTGGTCT
ATGACAGTTACCTTACCCCAAGGATGATGTGTGCTGGGGACCTTCGTGGGGGCAGAGACTCCTGCCAGGGAGACAGCGGC
GGGCCTCTTGTCTGTGAGCAGAACAACCGCTGGTACCTGCCAGGTGTCACCAGCTGGGGCGCAGCCTGTGGCCAGACAAA
CAAACCTGGTGTGTACACCAAAGTGACAGAAGTTCTTCCGTGGATTTACACCAACATGGAGAGCGAGGTGCCATTCAGAA
AATCCTAACCAGCTGGCCTGCTGCTCTGCACAGCACCGGCTGCTGTGACTAAGGGCGAATTC
```

Requested by: Gedrich, Richard on Tue

INCYTE PHARMACEUTICALS, INC.

SIGNED BY _____ **DATE** _____

WITNESSED AND UNDERST D BY Elizabeth C. Sullivan **DATE** _____

CROSS REFERENCES:

RB 55202

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EXHIBIT B

SUBJECT BLAST & NCBI

Purpose: Since I do not appear to have an in Frame start, I must re-do my BLASTS with the 5' end of my 147.3.3.49.28 clone (page 3) Human Est Data

NCBI BLAST Search Results BLAST Entrez ?

BLASTN 2.1.2

Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs". Nucleic Acids Res. 25:3389-3402.

RID: 975949296-29783-2804

Query= 147
(787 letters)

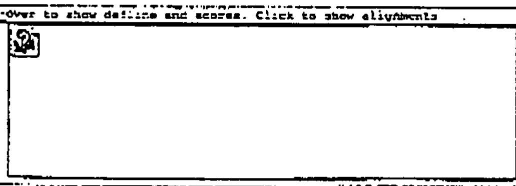
Database: GenBank Human EST entries
3,774,091 sequences; 1,183,000,365 total letters

If you have any problems or questions with the results of this search please refer to the BLAST FAQs

Taxonomy reports

Distribution of 26 Blast Hits on the Query Sequence

Mouse-over to show defline and scores. Click to show alignments



Sequences producing significant alignments:

		Score (bits)	E Value
gb|BE280394.1|BE280394	601158674F1 NIH_MGC_21 Homo sapiens ...	452	e-125
gb|BE732381.1|BE732381	601569601F1 NIH_MGC_21 Homo sapiens ...	291	3e-77
gb|BE073527.1|BE073527	RC5-BT0559-140200-011-H07 BT0559 Hom...	244	2e-62
gb|BF909673.1|BF909673	601501682F1 NIH_MGC_70 Homo sapiens ...	42	0.18
gb|AI138859.1|AI138859	qc04d10.x1 Soares_testis_NHT Homo sa...	42	0.18
gb|AI138858.1|AI138858	qc04d09.x1 Soares_testis_NHT Homo sa ...	42	0.18
gb|AA861735.1|AA861735	ak38b11.s1 Soares_testis_NHT Homo sa .	42	0.18
gb|AA197521.1|AA197521	zz...07.s1 Soares_testis_NHT Homo sa...	42	0.18
gb|AA193577.1|AA193577	zz72e07.r1 Soares_testis_NHT Homo sa...	42	0.18
gb|BF105146.1|BF105146	601622195F1 NIH_MGC_75 Homo sapiens ...	40	0.71
gb|BE903707.1|BE903707	601675007F1 NIH_MGC_21 Homo sapiens	38	2.8
gb|BE710507.1|BE710507	601562630F1 NIH_MGC_20 Homo sapiens ...	38	2.8
gb|BE158110.1|BE158110	MR2-HT0378-170200-202-a12 HT0378 Hom...	38	2.8
gb|BE158087.1|BE158087	MR2-HT0378-140200-202-a10 HT0378 Hom...	38	2.8
gb|AW190208.1|AW190208	x160a12.x1 NCI_CGAP_Pan1 Homo sapien...	38	2.8
gb|AI174367.1|AI174367	an17g03.s1 Gessler Wilms tumor Homo ...	38	2.8
gb|AI016056.1|AI016056	ov03h11.x1 NCI_CGAP_Kid3 Homo sapien...	38	2.8
gb|AA487804.1|AA487804	ab11e05.r1 Stratagene lung (#937210)...	38	2.8
gb|T82687.1|T82687	Hd459-1 Adult heart, Clontech Homo sapie...	38	2.8

Alignments

>gb|BE280394.1|BE280394 601158674F1 NIH_MGC_21 Homo sapiens cDNA clone IMAGE:3505123 5
Length = 751

Score = 452 bits (228), Expect = e-125
Identities = 349/376 (93%), Gaps = 10/376 (2%)
Strand = Plus / Plus

```
Query: 191 [illegible] 250
Sbjct: 86  gcatctccagcccaggcatctccagctgggacaccxccaggccgggcatctccagcccag 145

Query: 251 [illegible] 309
Sbjct: 146 [illegible] 205

Query: 310 [illegible] 369
Sbjct: 206 [illegible] 265

Query: 370 [illegible] 429
Sbjct: 266 [illegible] 325

Query: 430 [illegible] 484
Sbjct: 326 [illegible] 385

Query: 485 [illegible] 541
Sbjct: 386 [illegible] 445

Query: 542 [illegible] 557
Sbjct: 446 gg-cacacaggacca 460
```

Score = 174 bits (88), Expect = 2e-41
Identities = 98/100 (98%), Gaps = 1/100 (1%)
Strand = Plus / Plus

```
Query: 7   [illegible] 65
Sbjct: 51  [illegible] 110

Query: 66  [illegible] 105
Sbjct: 111 [illegible] 150
```

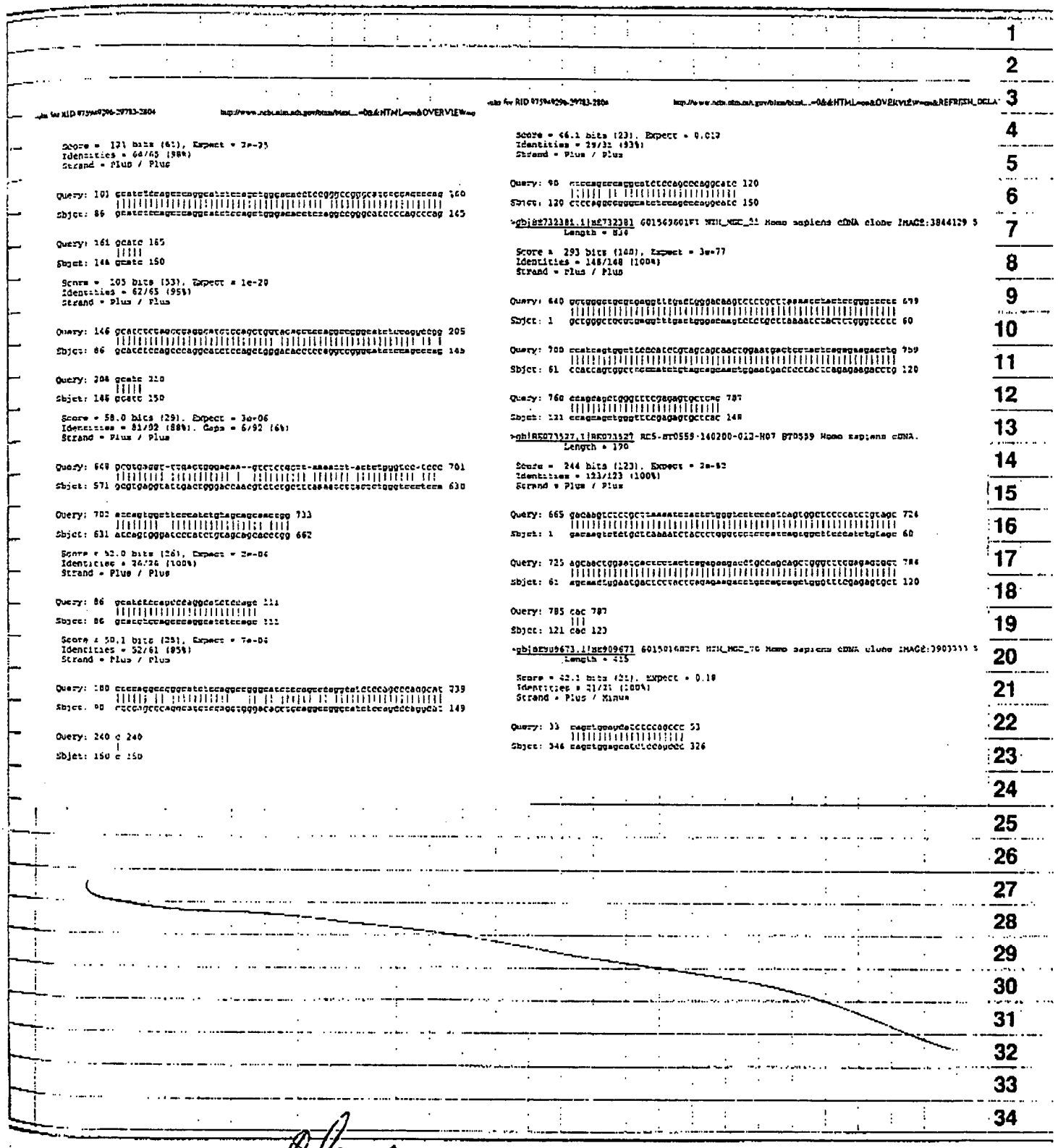
SIGNED BY [signature] **DATE**

WITNESSED AND UNDERSTOOD BY Elizabeth C. Sullivan **DATE**

CROSS REFERENCES:

BAYER CORPORATION

SUBJECT _____



SIGNED BY _____ **DATE** _____

WITNESSED AND UNDERSTOOD BY Elizabeth C Sullivan **DATE** _____

BAYER CORP RATION

RB55202

SUBJECT _____

1

>gb|BF105165.1|BF105165 601822195F1 NIH_MGC_7b Homo sapiens cDNA clone IMAGE:4042559 5
Length = 844

Score = 40.1 bits (20), Expect = 0.71
Identities = 20/20 (100%)
Strand = Plus / Plus

Query: 750 agaagagccctgccagcagcrg 769
Sbjct: 639 agaagagccctgccagcagctg 658

>gb|BE903702.1|BE903702 601475807F1 NIH_MGC_21 Homo sapiens cDNA clone IMAGE:3958056 5
Length = 1030

Score = 38.2 bits (19), Expect = 2.8
Identities = 19/19 (100%)
Strand = Plus / Minus

Query: 226 tccagcccaggcatcctcg 244
Sbjct: 360 tccagcccaggcatcctcg 342

>gb|BE730507.1|BE730507 601542630F1 NIH_MGC_70 Homo sapiens cDNA clone IMAGE:3832446 5
Length = 945

Score = 38.2 bits (19), Expect = 2.8
Identities = 19/19 (100%)
Strand = Plus / Minus

Query: 226 tccagcccaggcatcctcg 244
Sbjct: 756 tccagcccaggcatcctcg 738

>gb|BE158110.1|BE158110 HH2-HT0378-140200-702-a10 HT0378 Homo sapiens cDNA.
Length = 184

Score = 38.2 bits (19), Expect = 2.8
Identities = 19/19 (100%)
Strand = Plus / Plus

Query: 387 cagcacccagcaaccagggc 405
Sbjct: 143 cagcacccagcaaccagggc 160

>gb|BE158087.1|BE158087 HH2-HT0378-140200-702-a10 HT0378 Homo sapiens cDNA.
Length = 166

Score = 38.2 bits (19), Expect = 2.8
Identities = 19/19 (100%)
Strand = Plus / Plus

Query: 387 cagcacccagcaaccagggc 405
Sbjct: 142 cagcacccagcaaccagggc 160

>gb|AA190208.1|AA190208 xl60e12.xl NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGE:2679070
Length = 542

Score = 38.2 bits (19), Expect = 2.8
Identities = 19/19 (100%)

>gb|AI138859.1|AI138859 qe04d10.x1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:173
3'.
Length = 480

Score = 42.1 bits (21), Expect = 0.18
Identities = 21/21 (100%)
Strand = Plus / Minus

Query: 708 ggcttcccatctgtagcagca 728
Sbjct: 264 ggcttcccatctgtagcagca 244

>gb|AI138859.1|AI138859 qe04d09.x1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:173
3'.
Length = 482

Score = 42.1 bits (21), Expect = 0.18
Identities = 21/21 (100%)
Strand = Plus / Minus

Query: 708 ggcttcccatctgtagcagca 728
Sbjct: 264 ggcttcccatctgtagcagca 244

>gb|AA861735.1|AA861735 ak38b11.s1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:180
3'.
Length = 337

Score = 42.1 bits (21), Expect = 0.18
Identities = 21/21 (100%)
Strand = Plus / Minus

Query: 708 ggcttcccatctgtagcagca 728
Sbjct: 164 ggcttcccatctgtagcagca 144

>gb|AA397523.1|AA397523 zt72e07.s1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:727
3'.
Length = 299

Score = 42.1 bits (21), Expect = 0.18
Identities = 21/21 (100%)
Strand = Plus / Minus

Query: 708 ggcttcccatctgtagcagca 728
Sbjct: 169 ggcttcccatctgtagcagca 149

>gb|AA397577.1|AA397577 zt72e07.r1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:727
5'.
Length = 558

Score = 42.1 bits (21), Expect = 0.18
Identities = 21/21 (100%)
Strand = Plus / Plus

Query: 708 ggcttcccatctgtagcagca 728
Sbjct: 496 ggcttcccatctgtagcagca 516

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CROSS REFERENCES:

RB55202 23

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SUBJECT ____________________

```
sts for RID 973940296-29783-2604        http://www.ncbi.nlm.nih.gov/blast/blast ... =04&HTML=on&OVERVIEW=on&REFRESH_DELAY

 Strand = Plus / Plus

Query: 253 atcactttccaggtcctca 271
           |||||||||||||||||||
Sbjct: 50  atcactttccaggtcctca 68

>gb|AI176167.1|AI176167 an17g02.x1 Gessler Wilms tumor Homo sapiens cDNA clone
          IMAGE:1698962 3' similar to gb:M64716 40S RIBOSOMAL
          PROTEIN S25 (HUMAN);contains Alu repetitive element;.
          Length = 396

 Score = 38.2 bits (19), Expect = 2.8
 Identities = 25/27 (92%)
 Strand = Plus / Plus

Query: 33 cagctggagcatctccagcccaggtat 59
          ||||||||||||||||| ||| |||||
Sbjct: 64 cagctggagcatctccacccctggtat 90

>gb|AI016056.1|AI016056 ov02h11.x1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:1636197
           similar to TR:O14671 O14671 LNMAK. [1] ;.
          Length = 450

 Score = 38.2 bits (19), Expect = 2.8
 Identities = 19/19 (100%)
 Strand = Plus / Plus

Query: 158 caggcatccccagcctggta 176
           ||||||||||||||||||
Sbjct: 399 caggcatccccagcctggta 417

>gb|AA487804.1|AA487804 ab11e05.r1 Stratagene lung (#937210) Homo sapiens cDNA clone
           IMAGE:840222 5' similar to contains MER1.b1 MER1
           repetitive element ;.
          Length = 339

 Score = 38.2 bits (19), Expect = 2.8
 Identities = 22/23 (95%)
 Strand = Plus / Minus

Query: 375 ctcctgccaggtcagcaccagca 397
           ||||||||||||||||| |||||
Sbjct: 225 ctcctgccaggtcagcaccagca 203

>gb|T82687.1|T82687 Hd659-f Adult heart, Clontech Homo sapiens cDNA clone d659-f.
          Length = 208

 Score = 38.2 bits (19), Expect = 2.8
 Identities = 19/19 (100%)
 Strand = Plus / Plus

Query: 19  cagcatccccagcccaggt 37
           |||||||||||||||||||
Sbjct: 96  gagcatccccagcccaggt 114

  Database: GenBank Human EST entries
    Posted date:              7:52 PM
  Number of letters in database: 1,183,000,365
  Number of sequences in database: 2,774,091

sts for RID 973940296-29783-2604        http://www.ncbi.nlm.nih.gov/blast/blast ... =04&HTML=on&OVERVIEW=on&REFRESH_DELAY

Lambda     K      H
   1.37    0.711     1.31

Gapped
Lambda     K      H
   1.37    0.711     1.31

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 710245
Number of Sequences: 2774091
Number of extensions: 710245
Number of successful extensions: 194161
Number of sequences better than 10.0: 28
length of query: 787
length of database: 1,183,000,365
effective HSP length: 20
effective length of query: 767
effective length of database: 1,127,518,545
effective search space: 864806724015
effective search space used: 864806724015
T: 0
A: 0
X1: 6 (11.9 bits)
X2: 10 (19.8 bits)
S1: 12 (24.3 bits)
S2: 19 (38.2 bits)
```

Conclusion:

- The 33.49.28 sequence hits BE782381 which I already knew hit.

- It also overlaps with BE280394 for 726 nucleotides. BE280394 has an additional 51 nt at its 5' end.

- I will translate it to see if it contains an in frame ATG.

SIGNED BY [signature] **DATE** ____

WITNESSED AND UNDERSTOOD BY [signature] **DATE**

BAYER CORPORATION

RB55202

BE280394 GenBank Report

EXHIBIT tabbies

SUBJECT

NCBI Nucleotide

PubMed | Nucleotide | Protein | Genome | Structure | PopSet | Taxonomy | OMIM

Search Nucleotide for [ ] Go | Clear

Limits | Index | History | Clipboard

Display Default | View as HTML | Save | Add to Clipboard | Hide Brief and Lis

1: GI "9155397" [EST] 601158674F1 NIH_MGC_21 Homo... Taxonomy

IDENTIFIERS

dbEST Id: 9653095
EST name: 601158674F1
GenBank Acc: BE280394
GenBank gi: 9155397

CLONE INFO

Clone Id: IMAGE:3505123 (5')
Plate: LLCtx181 Row: d Column: 20
DNA type: cDNA

PRIMERS

PolyA Tail: Unknown

SEQUENCE

AACCCTCGACGGACTCTTCCCCTCAGAGACCATGGAGAGGGACACCCACGCGAATCCATCT
CCAGCAAGAACACCTTCAGACTGCAGCATCTCCAGCCCAGGCATCTCCAGCTGGGACACC
TCCAGGCTGGGCATCTCCAGCCCAGGCATCACTTTCCAGG[illegible]
CATCTGCCAGGTCAGCTCGGTGACAACCTCCCAACCAGAGTGTACCTTGTTAGAGCAA
CACCAGTGGGGGTGTACCCATCCGATCATCTGCTGCCAGGTCAGCACGAGCAAGCCAGGC
GCACAGTGGAGAGCGCAGGTACGAGCCTGACCAAGTTCAACTGAGCAGGGACGCCCAGAA
GCAGCTACCCACTCATCGCACTGCAGTGCTCACTCCTCATTCCCCTGGATGGTTTACGCT
CATCATCCTCTTCCAGTTCTGCCAGCGCACACAGCGATCAAGGTCACAAGGACCAAGATG
TGTGAGAGCTTGTCCCAAAGCACGCCTGTTCGCTTGTGCACGGGTGTATGGGACTTCCA
AAGACTGAAGAGTTGACAGACGCTGTGCTAGCGTGAGGTATTCACTGGGACCAACGTCTC
TGCTTTAAAAGTTACTCTGGTTCCCTCCAATCAGTGGGATCCCATGTGTAGCAGCACCT
GGGAATTGACTCTACTACAGAGAAGACTGCCAGCGAGTGGGATCAAAGACCTCCCCGGA
CACGACGTGCCCACAGCATTCGCAAAGATTA

Quality: High quality sequence starts at base: 6
Quality: High quality sequence stops at base: 513

Entry Created:
Last Updated:

COMMENTS

Tissue Procurement: ATCC
cDNA Library Preparation: Ling Meng/Rubin Laboratory
cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
DNA Sequencing by: Incyte Genomics, Inc.
Clone distribution: MGC clone distribution information can be found through the I.M.A.G.E. Consortium/LLNL at: image.llnl.gov

LIBRARY

Lib Name: NIH_MGC_21
Organism: Homo sapiens
Organ: placenta

1 of 2 12/4/2000 [illegible]

Tissue type: choriocarcinoma
Lab Host: DH10B (phage-resistant)
Vector: pOTB7
R. Site 1: XhoI
R. Site 2: SfiI
Description: cDNA made by oligo-dT priming. Directionally cloned into EcoRI/XhoI sites using the following 5' adaptor: GGCACGAG(G)5. Size-selected >500bp for average insert size 1.8kb. Library constructed by Ling Meng in the laboratory of Gerald M. Rubin (University of California, Berkeley) using ZAP-cDNA synthesis kit (Stratagene) and Superscript II RT (Life Technologies).

SUBMITTER

Name: Robert Strausberg, Ph.D.
Tel: (301) 496-1550
E-mail: Robert_Strausberg@nih.gov

CITATIONS

Title: National Institutes of Health, Mammalian Gene Collection (MGC)
Authors: NIH-MGC http://www.ncbi.nlm.nih.gov/MGC/
Year:
Status: Unpublished

MAP DATA

Restrictions on Use | Write to the HelpDesk
NCBI | NLM | NIH

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WITNESSED AND UNDERSTOOD BY Elizabeth C. Sullivan DATE

CROSS REFERENCE:

BAYER CORPORATION

EXHIBIT tabbies D

SUBJECT _____

Translation Results

LifeTools — Translation Results

Interactive:

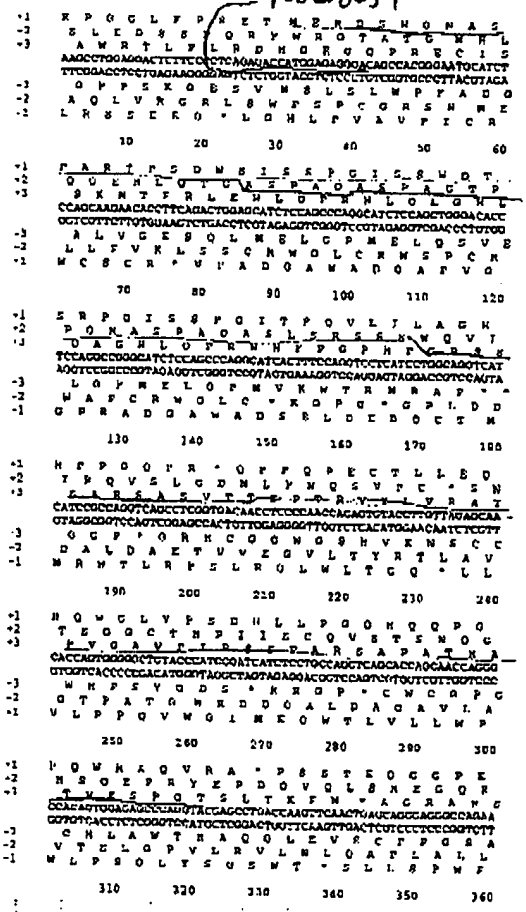
LifeTools:

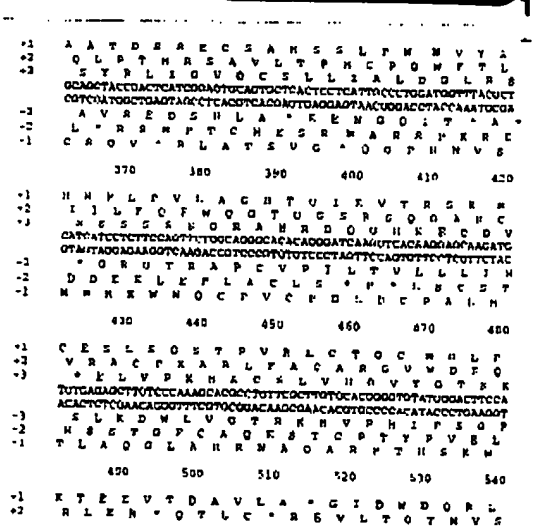
Confidential – Property of Incyte Genomics, Inc. LifeTools Version 3.1 SunServer

coolEST147:HE280394:2

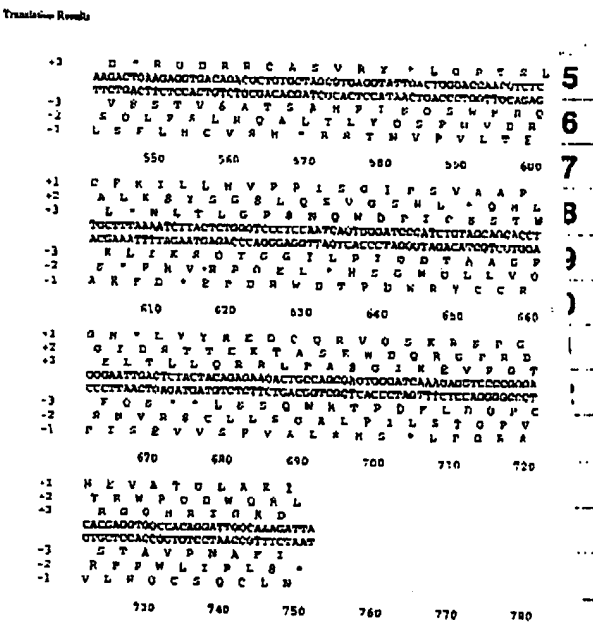
Using (Standard Code) Genetic Code; Translating cDNA
Color Scheme: Met (M) -- Stop (*)

Sequence: coolEST147:HE280394:2 R6L0054





Translation Results



28
29
30
31
32
33
34

SIGNED BY _____ DATE ___

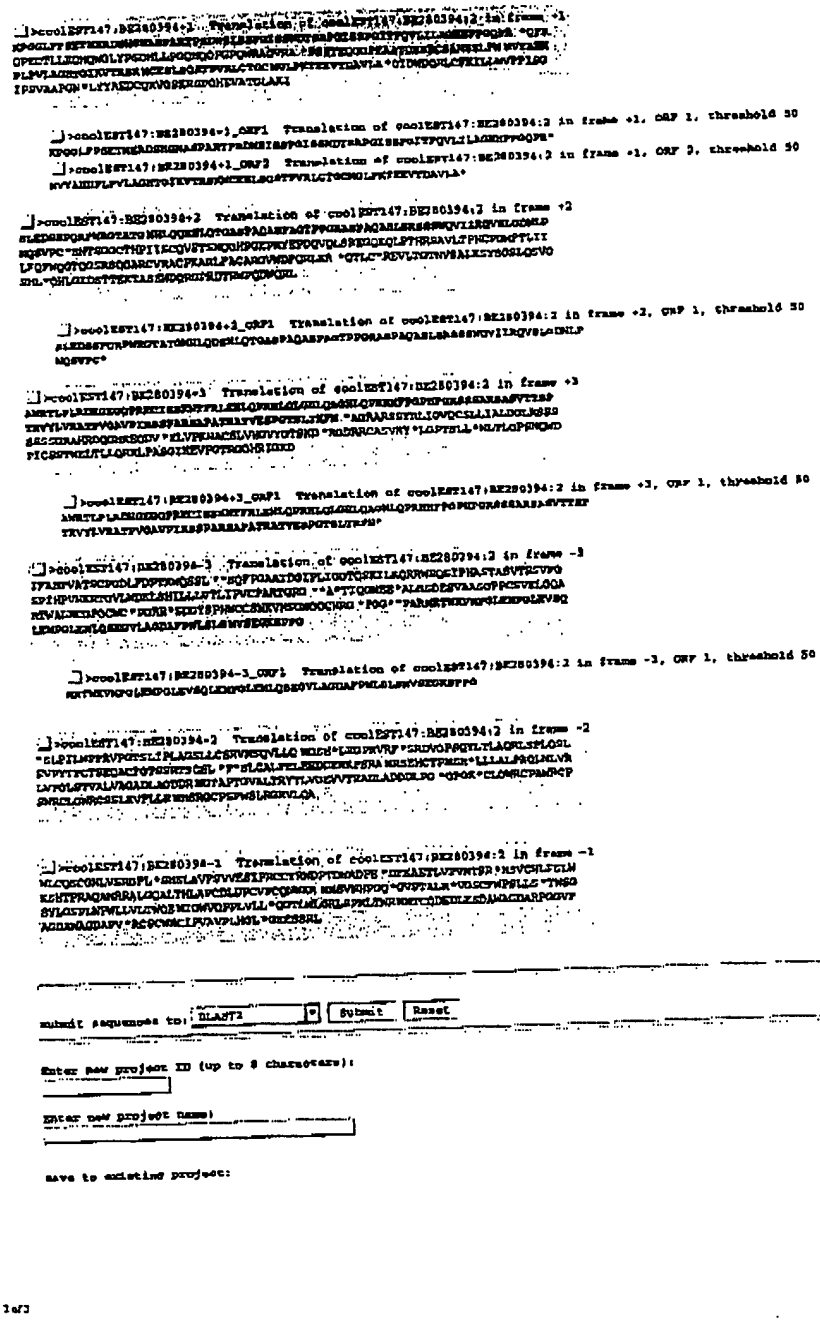
WITNESSED AND UNDERSTOOD BY Elizabeth C Sullivan DATE ___

CROSS REFERENCES:

BAYER CORPORATION

RB5202

SUBJECT _____



Conclusion: Underlined sequence on P 27 Aligns with my coding region Open Reading Frame corresponds to Base insertion in the BE280394 Seq seen on the BLAST analysis

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CROSS REFERENCES:

BAYER CORPORATION

SUBJECT

Conclusions (continued)

* MERDSH appears to be what I had Looked

The ATG here looks to be a great Kozak consensus

ACCATGG.

This is it.

= ORDER Primers

LBRI #147 amino acid Sequence

MERDSHGNASPARTPSAGASPAQVSPAGTPPGRASPAQASPAQASPAGTPPGRASPAQASPAGTPPGRASPG
RASPAQASPAQASPALASLSRSSSGRSSSARSASVTTSPTRVYLVRATPVGAVPIRSSPARSAPATRATRES
PGTSLPKFTWREGQKQLPLIGCVLLLIALVVSLIILFQFWQGHTGIRYKEQRESCPKHAVRCDGVVDCKLKS
DELGCVRFDWDKSLLKIYSGSSHQWLPICSSNWNDSYSEKTCQQLGFESAHRTTEVAHRDFANSFSILRYNS
TIQESLHRSECPSQRYISLQCSHCGLRAMTGRIVGGALASDSKWPWQVSLHFGTTHICGGTLIDAQWVLTAA
HCFFVTREKVLEGWKVYAGTSNLHQLPEAASIAEIIINSNYTDEEDDYDIALMRLSKPLTLSAHIHPACLPM
HGQTFSLNETCWITGFGKTRETDDKTSPFLREVQVNLIDFKKCNDYLVYDSYLTPRMMCAGDLRGGRDSCQG
DSGGPLVCEQNNRWYLAGVTSWGAGCGQRNKPGVYTKVTEVLPWIYSKMESEVRFRKS

Start is an NcoI site.
My predicted sequence diverges from the 5' end of BE280394 and a consensus splice acceptor (CAG).

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CROSS REFERENCES:

BAYER CORPORATION

RBS5202

EXHIBIT E

SUBJECT Oligo Order

LBRI Oligos

CoolEST 147- oligos to MY predicted genomic sequence

RGL0054
Cloning primer 23-mer to BE280394 sequence

5' CTC AGA GAC CAT GGA GAG GGA CA 3'

RGL0055
Sense Sequencing Primer 21-mer; bases 331-351 of 147.33.49.28contig2

5' CAA CCA GAG TGT ACC TTG TTA 3'

RGL0056
antiense Sequencing Primer 20-mer; bases 441-460 of 147.33.49.28contig2

5' CAG GTG AAC TTG GGC AGG CT 3'

Orbit

oligos to clone "Full-length 147" shown on Page 29

SIGNED BY _____ DATE _____

WITNESSED AND UNDERST D BY Elizabeth C Sullivan DATE

CR SS REFERENCES:

BAYER CORPORATION

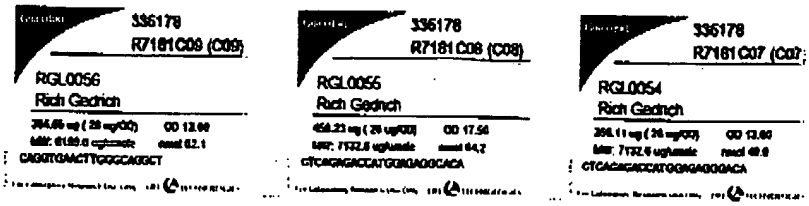
RB55202

EXHIBIT tabbies F

SUBJECT PCR with RGL0054/0028

Purpose: to test hypothesis for the new 5' ends

Reciprocal oligos



Resuspended RGL0054 to 200uM
with 250ul dH2O
dilute 1/4 to 50ul (25ul + 75ul dH2O)
in 50mM for PCR

2 RNS
do placenta cDNA -/+ RT
use Advantage GC kit (Clontech)

RNS
1) -RT
2) +RT

RXN	3rxn (3)	
2ul placenta cDNA	-	1) Aliq + PM
10ul 5X BLP	30ul	2) Add cDNA
1ul Advantage GC polymerase	3ul	3) Cycle: PCS600
1ul 10mM dNTP	3ul	
1ul RGL0054	3ul	94°C 1' → {94°C 15" → 68°C 3.5'} → 72°C 7' ∞
1ul RGL0028	3ul	1 cycle {35 cycles} 1 cycle
5ul GC melt	15ul	
29ul dH2O	87ul	
50ul	48ul/RXN	

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CROSS REFERENCES:

BAYER CORPORATION

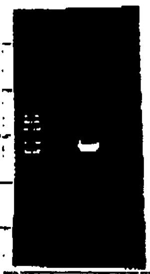
EXHIBIT G

SUBJECT

√ 5 µl

Ran sample on 1.2% Agarose gel (1xTAE)

M = 1kb Ladder (GIBCO)



The Run worked. An ~1.8Kb Band was seen in the +RT Run only

Clone into pCRII

Ran Remainder of the sample on a 1.2% Agarose gel

Cut out the Fragment

QIAquick Gel Extraction Kit Protocol

using a microcentrifuge

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low melt agarose gels in TAE or TBE buffer.

Notes:
- NEW The yellow color of Buffer QG indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Isopropanol (100%) will be required
- A heating block or water bath at 50°C is required.
- All centrifugation steps are carried out at ≥10,000 x g (~13,000 rpm) in a conventional table-top microcentrifuge.
- 3M sodium acetate, pH 5.0, may be necessary.

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.
2. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl).**
   For example, add 300 µl of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.
3. **Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.**
   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.
4. **After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose).**
   If the color of the mixture is orange or violet, add 10 µl of 3M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
   The adsorption of DNA to QIAquick membrane is efficient only at pH ≤7.5. Buffer QG contains a pH indicator which is yellow at pH ≤7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.
5. **Add 1 gel volume of isopropanol to the sample and mix.**
   For example, if the agarose gel slice is 100 mg, add 100 µl isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.
6. **Place a QIAquick spin column in a provided 2-ml collection tube.**
7. **To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.**
   The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
8. **Discard flow-through and place QIAquick column back in the same collection tube.**
   Collection tubes are reused to reduce plastic consumption.
9. **(Optional): Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.**
   This step will remove all traces of agarose. It is only required for direct sequencing, in vitro transcription or microinjection.
10. **To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min.**
    Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE, before centrifuging.
11. **Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at ≥10,000 x g (~13,000 rpm).**
    IMPORTANT. Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
12. **Place QIAquick column into a clean 1.5-ml microfuge tube.**
13. **To elute DNA, add 50 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) or H_2O to the center of the QIAquick column and centrifuge for 1 min at maximum speed. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick column, let stand for 1 min, and then centrifuge for 1 min.**
    IMPORTANT. Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl.
    Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

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WITNESSED AND UNDERSTOOD BY Elizabeth C Sullivan DATE

CROSS REFERENCES:

BAYER CORPORATION

RB55202

EXHIBIT H

SUBJECT

Eluted in 36μl dH2O 147 0054/78 PCR

TA cloning kit (Invitrogen)

Ligate Frag into pCRII

Rxn

1μl vector

1μl 10X

4μl dH2O

1μl Ligase

3μl Frag

10μl

16°C O/N Ti: 550pm

Transform TOP10

2μl Rxn ~~[illegible]~~ tube

- ice 25'
- 42°C 30"
- ice 2'

add 400μl SOC

37°C 40'

plate 100μl on LB+Amp Plate + Bluogal

37°C O/N T/

SIGNED BY [signature] DATE

WITNESSED AND UNDERST OD BY Elizabeth C Sullivan DATE

CR SS REFERENCE :

BAYER CORPORATION

EXHIBIT tabbies I

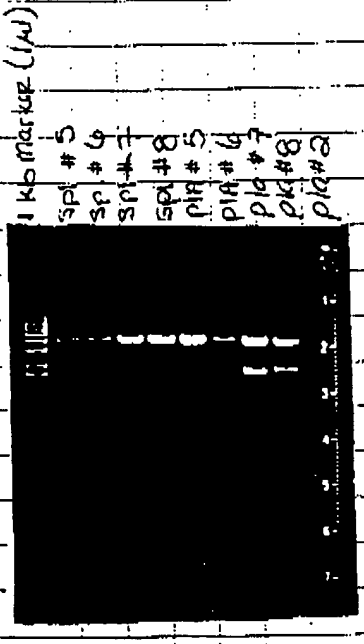
SUBJECT LBRI #147

① miniprep of Spl 5,6,7,8 : pla 5,6,7,8 : pla 2
* Followed same protocol from page 1 of this notebook.
* Eluted DNA in 50µl water

② Restriction enzyme digestion of pla 5,6,7,8, Spl 5,6,7,8 : pla 2

Plasmid	3µl	Digest for 2hrs @ 37°C
EcoRI	1µl	
Buffer 2	2µl	
H_2O	14µl	
	20µl	

③ Ran 1% Agarose Gel



* The miniprep from pla2 gave negative results. Therefore, I did a new transformation using 1µl of a 1:200 dilution of my original pla2 miniprep. Transformed in 30µ of DH5α max efficiency.

- 1µ dilution + 30µ cells
- incubate on ice for 5-10 minutes
- Add 100µ SOC
- Plate 100µ on Amp plates
- Incubate @ 37°C overnight

SIGNED BY Lisa Parker DATE

WITNESSED AND UNDERSTOOD BY Dwayne A. Dekker DATE

BAYER CORPORATION

SUBJECT LBRI #147

① Miniprep of #147 5428 (LBRI)
Rich Gedrich gave me 4 clones from Spleen
and 4 clones from placenta

② I followed the following protocol using 1.5ml culture

QIAprep Spin

QIAprep Spin Miniprep Kit Protocol

using a microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5-ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 31.

! Please read Important Notes for QIAprep Procedures on pages 14–15 before starting.

Procedure

1. Resuspend pelleted bacterial cells in 250 µl of Buffer P1 and transfer to a microfuge tube.
   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
2. Add 250 µl of Buffer P2 and gently invert the tube 4–6 times to mix.
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
3. Add 350 µl of Buffer N3 and invert the tube immediately but gently 4–6 times.
   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.
4. Centrifuge for 10 min.
   A compact white pellet will form.
   During centrifugation, place a QIAprep spin column in a 2-ml collection tube.
5. Apply the supernatants from step 4 to the QIAprep column by decanting or pipetting.
6. Centrifuge 30–60 sec. Discard the flow-through.
7. (Optional): Wash QIAprep spin column by adding 0.5 ml of Buffer PB and centrifuging 30–60 sec. Discard the flow-through.
   This step is necessary to remove trace nuclease activity when using *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.
8. Wash QIAprep spin column by adding 0.75 ml of Buffer PE and centrifuging 30–60 sec.
9. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.
   ! IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. Place QIAprep column in a clean 1.5-ml microfuge tube. To elute DNA, add 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of each QIAprep column, let stand for 1 min, and centrifuge for 1 min.

QIAprep Spin Miniprep Kit Protocol

using 5-ml collection tubes

The QIAprep Spin Miniprep procedure can be performed using 5-ml centrifuge tubes (e.g., Greiner, Cat. No. 115101 or 115261) as collection tubes to decrease handling. The standard protocol on pages 18–19 should be followed with the following modifications:

Step 4: Place QIAprep spin column in a 5-ml centrifuge tube instead of a 2-ml collection tube.

Step 6: Centrifuge at 3000 x g for 1 min using a suitable rotor (e.g., Beckman® GS-6KR centrifuge at ~4000 rpm). (The flow-through does not need to be discarded).

Steps 7 & 8: For washing steps, centrifugation should be performed at 3000 x g for 1 min. (The flow-through does not need to be discarded).

Step 9: Transfer QIAprep column to a microfuge tube. Microcentrifuge at maximum speed for 1 min. Continue with step 10 of the protocol.

+ Pellet cells at 10,000 rpm for 2 minutes
+ ~~→~~ Elute DNA in 50µl's Water instead of Buffer EB
next time I do a miniprep
+ Store excess bacterial culture in 4°C

SIGNED BY Lisa Parkyn DATE

WITNESSED AND UNDERSTOOD BY Jacqueline A. Dreher DATE

BAYER CORPORATION

RB5J846

EXHIBIT tabbies J

SUBJECT LBRI #147

1

2 ① Selected 2 pla2 clones & cultured overnight in

3 3mL LB + 1.5carb @ 37°C

4

5 ② Sent the following clones for sequencing:

6

7 Lisa Parkyn 03:35 PM

8 To: Janice Jackson/WESTH/PH/US/BAYER@BAYER-US-NOTES, Gwenda Ligon/WESTH/PH/US/BAYER@BAYER-US-NOTES

9 cc: David Eustice/WESTH/PH/US/BAYER@BAYER-US-NOTES

10 Subject: LBRI Sequencing

11

12 Hi Jan and Gwen,

13 These are for the LBRI #147 program.

14 The vector is pCRII . The host was Top10 and the preps were done with the Qiagen kit.

15 Thanks,

16 Lisa

spl5, spl6, pla6, pla2

3λ plasmid
2λ primer
7λ H2O

pla7, pla8

1λ plasmid
2λ primer
9λ H2O

Primers

M13F, RG029, 032, 033, 034, 047, 052, M13R

17
1. RG_spl5_M13F
2. RG_spl5_029
3. RG_spl5_032
4. RG_spl5_033
5. RG_spl5_034
6. RG_spl5_047
7. RG_spl5_052
8. RG_spl5_M13R
9. RG_spl6_M13F
10. RG_spl6_029
11. RG_spl6_032
12. RG_spl6_033
13. RG_spl6_034
14. RG_spl6_047
15. RG_spl6_052
16. RG_spl6_M13R
17. RG_pla6_M13F
18. RG_pla6_029
19. RG_pla6_032
20. RG_pla6_033
21. RG_pla6_034
22. RG_pla6_047
23. RG_pla6_052
24. RG_pla6_M13R
25. RG_pla7_M13F
26. RG_pla7_029
27. RG_pla7_032
28. RG_pla7_033
29. RG_pla7_034
30. RG_pla7_047
31. RG_pla7_052
32. RG_pla7_M13R
33. RG_pla8_M13F
34. RG_pla8_029
35. RG_pla8_032
36. RG_pla8_033
37. RG_pla8_034
38. RG_pla8_047
39. RG_pla8_052
40. RG_pla3_M13R
41. RG_pla3_M13F
42. RG_pla3_029
43. RG_pla3_032
44. RG_pla3_033
45. RG_pla3_034
46. RG_pla3_047
47. RG_pla3_052
48. RG_pla3_M13R

34

SIGNED BY Lisa Parkyn DATE

WITNESSED AND UNDERST OD BY Dwynwen A-DeHuer DATE

CROSS REFERENCES:

BAYER CORPORATION

RB558 46

EXHIBIT tabbies K

SUBJECT LBRI #147

① LifeTools ClustalW results comparing coolEST147_147spl5_2 to the 147 consensus sequence (DNA sequence) (pSE vector)

Confidential – Property of Incyte Genomics, Inc. LifeTools Version 3.1 SeqServer

coolEST147:147spl5:2
coolEST147:147FLconsensus:2

CLUSTAL W (1.7) Multiple Sequence Alignments

```
Sequence format is Pearson
Sequence 1: coolEST147_147spl5_2          1767 bp
Sequence 2: coolEST147_147FLconsensus_2   1748 bp
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score:  100
Start of Multiple Alignment
There are 1 groups
Aligning...
Group 1: Sequences:   2      Score:33212
Alignment Score 13534
CLUSTAL-Alignment file created  [baaa03540.aln]
CLUSTAL W (1.7) multiple sequence alignment

coolEST147_147spl5_2          GAATTCGGCTCAGAGACCATGGAGAGGGACAGCCACGGGAATGCATCTCC
coolEST147_147FLconsensus_2   --------CTCAGAGACCATGGAGAGGGACAGCCACGGGAATGCATCTCC
                                        ****************************************

coolEST147_147spl5_2          AGCAAGAACACCTTCAGCTGGAGCATCTCCAGCCCAGGCATCTCCAGCTG
coolEST147_147FLconsensus_2   AGCAAGAACACCTTCAGCTGGAGCATCTCCAGCCCAGGCATCTCCAGCTG
                              ****************************************************

coolEST147_147spl5_2          GGACACCTCCAGGCCGGGGCATCTCCAGCCCAGGCATCTCCAGCCCAGGCA
coolEST147_147FLconsensus_2   GGACACCTCCAGGCCGGGGCATCTCCAGCCCAGGCATCTCCAGCCCAGGCA
                              ****************************************************

coolEST147_147spl5_2          TCTCCAGCTGGGACACCTCCGGGCCGGGCATCTCCAGCCCAGGCATCTCC
coolEST147_147FLconsensus_2   TCTCCAGCTGGGACACCTCCGGGCCGGGCATCTCCACCCCAGGCATCTCC
                              ****************************************************

coolEST147_147spl5_2          AGCTGGTACACCTCCAGGCCGGGCATCTCCAGGCCGGGCATCTCCAGCCC
coolEST147_147FLconsensus_2   AGCTGGTACACCTCCAGGCCGGGCATCTCCAGGCCGGGCATCTCCAGCCC
                              ****************************************************

coolEST147_147spl5_2          AGGCATCTCCAGCCCGGGCATCTCCGGCTCTGGCATCACTTTCCAGGTCC
coolEST147_147FLconsensus_2   AGGCATCTCCAGCCCGGGCATCTCCGGCTCTGGCATCACTTTCCAGGTCC
                              ****************************************************

coolEST147_147spl5_2          TCATCCGGCAGGTCATCATCCGCCAGGTCAGCCTCGGTGACAACCTCCCC
coolEST147_147FLconsensus_2   TCATCCGGCAGGTCATCATCCGCCAGGTCAGCCTCGGTGACAACCTCCCC
                              ****************************************************

coolEST147_147spl5_2          AACCAGAGTGTACCTTGTTAGAGCAACACCAGTGGGGGCTGTACCCATCC
coolEST147_147FLconsensus_2   AACCAGAGTGTACCTTGTTAGAGCAACACCAGTGGGGGCTGTACCCATCC
                              ****************************************************

coolEST147_147spl5_2          GATCATCTCCTGCCAGGTCAGCACCAGCAACCAGGGCCACCAGGGAGAGC
coolEST147_147FLconsensus_2   GATCATCTCCTGCCAGGTCAGCACCAGCAACCAGGGCCACCAGGGAGAGC
                              ****************************************************
```

*Continued on pg. 16

SIGNED BY Alisa Parker DATE

WITNESSED AND UNDERSTOOD BY Dwayne A. Dalton DATE

CROSS REFERENCES:

BAYER CORPORATION

RB55846

SUBJECT LBRI #147

LifeTools Clustal W... (DNA Sequence)

```
coolEST147_147spl5_2      CCAGGTACGAGCCTGCCCAAGTTCACCTGGCGGGAGGGCCAGAAGCAGCT
coolEST147_147FLconsensus_2 CCAGGTACGAGCCTGCCCAAGTTCACCTGGCGGGAGGGCCAGAAGCAGCT
                          ***************************************************

coolEST147_147spl5_2      ACCGCTCATCGGGTGCGTGCTCCTCCTCATTGCCCTGGTGGTTTCGCTCA
coolEST147_147FLconsensus_2 ACCGCTCATCGGGTGCGTGCTCCTCCTCATTGCCCTGGTGGTTTCGCTCA
                          ***************************************************

coolEST147_147spl5_2      TCATCCTCTTCCAGTTCTGGCAGGGCCACACAGGGATCAGGTACAAGGAG
coolEST147_147FLconsensus_2 TCATCCTCTTCCAGTTCTGGCAGGGCCACACAGGGATCAGGTACAAGGAG
                          ***************************************************

coolEST147_147spl5_2      CAGAGCGGAGAGCTGTCCCAAGCACGCTGTTCGCTGTGACGGGGTGGTGGA
coolEST147_147FLconsensus_2 CAGAGGGAGAGCTGTCCCAAGCACGCTGTTCGCTGTGACGGGGTGGTGGA
                          ***************************************************

coolEST147_147spl5_2      CTGCAAGCTGAAGAGTGACGAGCTGGGCTGCGTGAGGTTTGACTGGGACA
coolEST147_147FLconsensus_2 CTGCAAGCTGAAGAGTGACGAGCTGGGCTGCGTGAGGTTTGACTGGGACA
                          ***************************************************

coolEST147_147spl5_2      AGTCTCTGCTTAAAATCTACTCTGGGTCCTCCCATCAGTGGCTTCCCATC
coolEST147_147FLconsensus_2 AGTCTCTGCTTAAAATCTACTCTGGGTCCTCCCATCAGTGGCTTCCCATC
                          ***************************************************

coolEST147_147spl5_2      TGTAGCAGCAACTGGAATGACTCCTACTCAGAGAAGACCTGCCAGCAGCT
coolEST147_147FLconsensus_2 TGTAGCAGCAACTGGAATGACTCCTACTCAGAGAAGACCTGCCAGCAGCT
                          ***************************************************

coolEST147_147spl5_2      GGGTTTCGAGAGTGCTCACCGGACAACCGAGGTTGCCCACAGGGATTTTG
coolEST147_147FLconsensus_2 GGGTTTCGAGAGTGCTCACCGGACAACCGAGGTTGCCCACAGGGATTTTG
                          ***************************************************

coolEST147_147spl5_2      CCAACAGCTTCTCAATCTTGAGATACAACTCCACCATCCAGGAAAGCCTC
coolEST147_147FLconsensus_2 CCAACAGCTTCTCAATCTTGAGATACAACTCCACCATCCAGGAAAGCCTC
                          ***************************************************

coolEST147_147spl5_2      CACAGGTCTGAATGCCCTTCCCAGCGGTATATCTCCCTCCAGTGTTCCCA
coolEST147_147FLconsensus_2 CACAGGTCTGAATGCCCTTCCCAGCGGTATATCTCCCTCCAGTGTTCCCA
                          ***************************************************

coolEST147_147spl5_2      CTGCGGACTGAGGGCCATGACCGGGCGGATCGTGGGAGGGGCGCTGGCCT
coolEST147_147FLconsensus_2 CTGCGGACTGAGGGCCATGACCGGGCGGATCGTGGGAGGGGCGCTGGCCT
                          ***************************************************

coolEST147_147spl5_2      CGGATAGCAAGTGGCCTTGGCAAGTGAGTCTGCACTTCGGCACCACCCAC
coolEST147_147FLconsensus_2 CGGATAGCAAGTGGCCTTGGCAAGTGAGTCTGCACTTCGGCACCACCCAC
                          ***************************************************

coolEST147_147spl5_2      ATCTGTGGAGGCACGCTCATTGACGCCCAGTGGGTGCTCACTGCCGCCCA
coolEST147_147FLconsensus_2 ATCTGTGGAGGCACGCTCATTGACGCCCAGTGGGTGCTCACTGCCGCCCA
                          ***************************************************

coolEST147_147spl5_2      CTGCTTCTTCGTGACCCGGGAGAAGGTCCTGGAGGGCTGGAAGGTGTACG
coolEST147_147FLconsensus_2 CTGCTTCTTCGTGACCCGGGAGAAGGTCCTGGAGGGCTGGAAGGTGTACG
                          ***************************************************

coolEST147_147spl5_2      CGGGCACCAGCAACCTGCACCAGTTGCCTGAGCCAGCCTCCATTGCCGAG
coolEST147_147FLconsensus_2 CGGGCACCAGCAACCTGCACCAGTTGCCTGAGGCAGCCTCCATTGCCGAG
                          ***************************************************

coolEST147_147spl5_2      ATCATCATCAACAGCAATTACACCGATGAGGAGGACGACTATGACATCGC
coolEST147_147FLconsensus_2 ATCATCATCAACAGCAATTACACCGATGAGGAGGACGACTATGACATCGC
                          ***************************************************

coolEST147_147spl5_2      CCTCATGCGGCTGTCCAAGCCCCTGACCCTGTCCGCTCACATCCACCCTG
coolEST147_147FLconsensus_2 CCTCATGCGGCTGTCCAAGCCCCTGACCCTGTCCGCTCACATCCACCCTG
                          ***************************************************

coolEST147_147spl5_2      CTTGCCTCCCCATGCATGGACAGACCTTTAGCCTCAATGAGACCTGCTGG
coolEST147_147FLconsensus_2 CTTGCCTCCCCATGCATGGACAGACCTTTAGCCTCAATGAGACCTGCTGG
                          ***************************************************
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continued on pg. 17.

SIGNED BY Lisa Parkyn DATE

WITNESSED AND UNDERST OD BY Suzanne A Diller DATE

CROSS REFERENCES:

RB5846 17

BAYER CORPORATION

SUBJECT LBRI #147

LifeTools Clustal W... (DNA Sequence)

```
coolEST147_147spl5_2        ATCACAGGCTTTGGCAAGACCAGGGAGACAGATGACAAGACATCCCCCTT
coolEST147_147FLconsensus_2 ATCACAGGCTTTGGCAAGACCAGGGAGACAGATGACAAGACATCCCCCTT
                            **************************************************

coolEST147_147spl5_2        CCTCCGGGAGGTGCAGGTCAATCTCATCGACTTCAAGAAATGCAATGACT
coolEST147_147FLconsensus_2 CCTCCGGGAGGTGCAGGTCAATCTCATCGACTTCAAGAAATGCAATGACT
                            **************************************************

coolEST147_147spl5_2        ACTTGGTCTATGACAGTTACCTTACCCCAAGGATGATGTGTGCTGGGGAC
coolEST147_147FLconsensus_2 ACTTGGTCTATGACAGTTACCTTACCCCAAGGATGATGTGTGCTGGGGAC
                            **************************************************

coolEST147_147spl5_2        CTTCGTGGGGCCAGAGACTCCTGCCAGGGAGACAGCGGGGGGCCTCTTGT
coolEST147_147FLconsensus_2 CTTCGTGGCGGCAGAGACTCCTGCCAGGGAGACAGCGGGGGGCCTCTTGT
                            **************************************************

coolEST147_147spl5_2        CTGTGAGCAGAACAACCGCTGGTACCTGGCAGGTGTCACCAGCTGGGGCA
coolEST147_147FLconsensus_2 CTGTGAGCAGAACAACCGCTGGTACCTGGCAGGTGTCACCAGCTGGGGCA
                            **************************************************

coolEST147_147spl5_2        CAGGCTGTGGCCAGAGAAACAAACCTGGTGTGTACACCAAAGTGACAGAA
coolEST147_147FLconsensus_2 CAGGCTGTGGCCAGAGAAACAAACCTGGTGTGTACACCAAAGTGACAGAA
                            **************************************************

coolEST147_147spl5_2        GTTCTTCCCTGGATTTACAGCAAGATGGAGAGCGAGGTCGATTCAGAAA
coolEST147_147FLconsensus_2 GTTCTTCCCTGGATTTACAGCAAGATGGAGAGCGAGGTCGATTCAGAAA
                            **************************************************

coolEST147_147spl5_2        ATCCTAACCAGCTGGCCTGCTGCTCTGCACAGCACCGGCTGCTGTGACTC
coolEST147_147FLconsensus_2 ATCCTAACCAGCTGGCCTGCTGCTCTGCACAGCACCGGCTGCTGTGACTC
                            **************************************************

coolEST147_147spl5_2        GAGAAAAAGCCGAATTC

coolEST147_147FLconsensus_2 GAGAAA----------
                            ******
```

Protein Sequence

```
Sequence format is Pearson
Sequence 1: coolEST147_147spl5+1_1      562 aa
Sequence 2: coolEST147_147FLconsensus_3 562 aa
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score:  100
Start of Multiple Alignment
There are 1 groups
Aligning...
Group 1: Sequences:   2      Score:7726
Alignment Score 3604
CLUSTAL-Alignment file created  [baaa03SM3.aln]
CLUSTAL W (1.7) multiple sequence alignment

coolEST147_147spl5+1_1      MERDSHGNASPARTPSAGASPAQASPAGTPPGRASPAQASPAQASPAGTP
coolEST147_147FLconsensus_3 MERDSHGNASPARTPSAGASPAQASPAGTPPGRASPAQASPAQASPAGTP
                            **************************************************

coolEST147_147spl5+1_1      PGRASPAQASPAGTPPGRASPGRASPAQASPARASPALASLSRSSSGRSS
coolEST147_147FLconsensus_3 PGRASPAQASPAGTPPGRASPGRASPAQASPARASPALASLSRSSSGRSS
                            **************************************************

coolEST147_147spl5+1_1      SARSASVTTSPTRVYLVRATPVGAVPIRSSPARSAPATRATRESPGTSLP
coolEST147_147FLconsensus_3 SARSASVTTSPTRVYLVRATPVGAVPIRSSPARSAPATRATRESPGTSLP
                            **************************************************
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*continued on Pg. 18

SIGNED BY Lisa Parham DATE

WITNESSED AND UNDERSTOOD BY Dwayne A. DeHler DATE

BAYER CORPORATION

EXHIBIT tabbies L

SUBJECT LBRI #147

LifeTools Clustal W... (DNA Sequence)

```
coolEST147_147sp15_2        ATCACAGGCTTTGGCAAGACCAGGGAGACAGATGACAAGACATCCCCCTT
coolEST147_147FLconsensus_2 ATCACAGGCTTTGGCAAGACCAGGGAGACAGATGACAAGACATCCCCCTT
                            **************************************************

coolEST147_147sp15_2        CCTCCGGGAGGTGCAGGTCAATCTCATCGACTTCAAGAAATGCAATGACT
coolEST147_147FLconsensus_2 CCTCCGGGAGGTGCAGGTCAATCTCATCGACTTCAAGAAATGCAATGACT
                            **************************************************

coolEST147_147sp15_2        ACTTGGTCTATGACAGTTACCTTACCCCAAGGATGATGTGTGCTGGGGAC
coolEST147_147FLconsensus_2 ACTTGGTCTATGACAGTTACCTTACCCCAAGGATGATGTGTGCTGGGGAC
                            **************************************************

coolEST147_147sp15_2        CTTCGTGGGGGCAGAGACTCCTGCCAGGGAGACACCGGGGGGCCTCTTGT
coolEST147_147FLconsensus_2 CTTCGTGGGGGCAGAGACTCCTGCCAGGGAGACAGCGGGGGGCCTCTTGT
                            **************************************************

coolEST147_147sp15_2        CTGTGAGCAGAACAACCGCTGGTACCTGGCAGGTGTCACCAGCTGGGGCA
coolEST147_147FLconsensus_2 CTGTGAGCAGAACAACCGCTGGTACCTGGCAGGTGTCACCAGCTGGGGCA
                            **************************************************

coolEST147_147sp15_2        CAGGCTGTGGCCAGAGAAACAAACCTGGTGTGTACACCAAAGTGACAGAA
coolEST147_147FLconsensus_2 CAGGCTGTGGCCAGAGAAACAAACCTGGTGTGTACACCAAAGTGACAGAA
                            **************************************************

coolEST147_147sp15_2        GTTCTTCCCTGGATTTACAGCAAGATGGAGAGCGAGGTGCGATTCAGAAA
coolEST147_147FLconsensus_2 GTTCTTCCCTGGATTTACAGCAAGATGGAGAGCGAGGTGCGATTCAGAAA
                            **************************************************

coolEST147_147sp15_2        ATCCTAACCAGCTGGCCTGCTGCTCTGCACAGCACCGGCTGCTGTGACTC
coolEST147_147FLconsensus_2 ATCCTAACCAGCTGGCCTGCTGCTCTGCACAGCACCGGCTGCTGTGACTC
                            **************************************************

coolEST147_147sp15_2        GAGAAAAAGCCGAATTC

coolEST147_147FLconsensus_2 GAGAAA----------
                            ******
```

Protein Sequence

```
Sequence format is Pearson
Sequence 1: coolEST147_147sp15+1_1      562 aa
Sequence 2: coolEST147_147FLconsensus_3 562 aa
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score:  100
Start of Multiple Alignment
There are 1 groups
Aligning...
Group 1: Sequences:   2      Score:7726
Alignment Score 3604
CLUSTAL-Alignment file created  [baaa01SM3.aln]
CLUSTAL W (1.7) multiple sequence alignment

coolEST147_147sp15+1_1      MERDSHGNASPARTPSAGASPAQASPAGTPPGRASPAQASPAQASPAGTP
coolEST147_147FLconsensus_3 MERDSHGNASPARTPSAGASPAQASPAGTPPGRASPAQASPAQASPAGTP
                            **************************************************

coolEST147_147sp15+1_1      PGRASPAQASPAGTPPGRASPGRASPAQASPARASPALASLSRSSSGRSS
coolEST147_147FLconsensus_3 PGRASPAQASPAGTPPGRASPGRASPAQASPARASPALASLSRSSSGRSS
                            **************************************************

coolEST147_147sp15+1_1      SARSASVTTSPTRVYLVRATPVGAVPIRSSPARSAPATRATRESPGTSLP
coolEST147_147FLconsensus_3 SARSASVTTSPTRVYLVRATPVGAVPIRSSPARSAPATRATRESPGTSLP
                            **************************************************
```

*continued on pg. 18

SIGNED BY Lisa Parker DATE

WITNESSED AND UNDERSTOOD BY Dwayne A. DeHler DATE

RB55846

BAYER CORPORATION

SUBJECT LBRI #147

Protein sequence (Lifetools)

```
coolEST147_147spl5+1_1     KFTWREGQKQLPLIGCVLLLIALVVSLIILFQFWQGHTGIRYKEQRESCP
coolEST147_147FLconsensus_3 KFTWREGQKQLPLIGCVLLLIALVVSLIILFQFWQGHTGIRYKEQRESCP
                           **************************************************

coolEST147_147spl5+1_1     KHAVRCDGVVDCKLKSDELGCVRFDWDKSLLKIYSGSSHQWLPICSSNWN
coolEST147_147FLconsensus_3 KHAVRCDGVVDCKLKSDELGCVRFDWDKSLLKIYSGSSHQWLPICSSNWN
                           **************************************************

coolEST147_147spl5+1_1     DSYSEKTCQQLGFESAHRTTEVAHRDFANSFSILRYNSTIQESLHRSECP
coolEST147_147FLconsensus_3 DSYSEKTCQQLGFESAHRTTEVAHRDFANSFSILRYNSTIQESLHRSECP
                           **************************************************

coolEST147_147spl5+1_1     SQRYISLQCSHCGLRAMTGRIVGGALASDSKWPWQVSLHFGTTHICGGTL
coolEST147_147FLconsensus_3 SQRYISLQCSHCGLRAMTGRIVGGALASDSKWPWQVSLHFGTTHICGGTL
                           **************************************************

coolEST147_147spl5+1_1     IDAQWVLTAAHCFFVTREKVLEGWKVYAGTSNLHQLPEAASIAEIIINSN
coolEST147_147FLconsensus_3 IDAQWVLTAAHCFFVTREKVLEGWKVYAGTSNLHQLPEAASIAEIIINSN
                           **************************************************

coolEST147_147spl5+1_1     YTDEEDDYDIALMRLSKPLTLSAHIHPACLPMHGQTFSLNETCWITGFGK
coolEST147_147FLconsensus_3 YTDEEDDYDIALMRLSKPLTLSAHIHPACLPMHGQTFSLNETCWITGFGK
                           **************************************************

coolEST147_147spl5+1_1     TRETDDKTSPFLREVQVNLIDFKKCNDYLVYDSYLTPRMMCAGDLRGGRD
coolEST147_147FLconsensus_3 TRETDDKTSPFLREVQVNLIDFKKCNDYLVYDSYLTPRMMCAGDLRGGRD
                           **************************************************

coolEST147_147spl5+1_1     SCQGDSGGPLVCEQNNRWYLAGVTSWGTGCGQRNKPGVYTKVTEVLPWIY
coolEST147_147FLconsensus_3 SCQGDSGGPLVCEQNNRWYLAGVTSWGTGCGQRNKPGVYTKVTEVLPWIY
                           **************************************************

coolEST147_147spl5+1_1     SKMESEVRFRKS
coolEST147_147FLconsensus_3 SKMESEVRFRKS
                           ************
```

SIGNED BY Lisa Pavam DATE

WITNESSED AND UNDERSTOOD BY Dwayne A. Deliz DATE

CROSS REFERENCES: